



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C12N 15/82, 15/63, A01H 5/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/01563</b> <b>(43) International Publication Date:</b> 14 January 1999 (14.01.99)
<b>(21) International Application Number:</b> PCT/EP98/04171 <b>(22) International Filing Date:</b> 29 June 1998 (29.06.98) <b>(30) Priority Data:</b> 97201990.5 30 June 1997 (30.06.97) EP <b>(34) Countries for which the regional or international application was filed:</b> NL et al. <b>(71) Applicant (for all designated States except US):</b> MOGEN INTERNATIONAL N.V. [NL/NL]; Einsteinweg 97, NL-2333 CB Leiden (NL). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> STUIVER, Maarten, Hendrik [NL/NL]; Groenhoevelaan 71, NL-2343 BR Oegstgeest (NL). PONSTEIN, Anne, Silene [NL/NL]; Meerforel 5, NL-2318 MR Leiden (NL). OHL, Stephan, Andreas [DE/NL]; Magda Janssenspad 34, NL-2331 JP Leiden (NL). GODDIJN, Oscar, Johanna, Maria [NL/NL]; Oude Herengracht 5, NL-2312 LN Leiden (NL). SIMONS, Lambertus, Henricus [NL/NL]; Anne de Vrieslaan 20, NL-1187 WL Amsterdam (NL). DEKKER, Bernardus, Martinus, Maria [NL/NL]; Helena Hoeve 40, NL-2804 HZ Gouda (NL). HOEKSTRA, Sietske [NL/NL]; Groenhoevelaan 71, NL-2343 BR Oegstgeest (NL). TIGELAAR, Hen-		drik [NL/NL]; Onafhankelijkheidsweg 19, NL-2332 ZM Leiden (NL). ELZINGA, Nicolas [NL/NL]; Vianenstraat 200, NL-1106 DE Amsterdam (NL). <b>(74) Agent:</b> VAN WEZENBEEK, L., A., C., M.; Mogen International N.V., Einsteinweg 97, NL-2333 CB Leiden (NL). <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PLASMIDS FOR PLANT TRANSFORMATION AND METHOD FOR USING THE SAME <b>(57) Abstract</b> <p>This invention relates to the <i>Agrobacterium</i> mediated plant transformation, especially to transformation of plants with T-DNA, where read-through at the borders is prohibited. This can be done by inhibition of transfer of vector DNA to the plant cell by creating DNA binding sites outside the T-borders, but also to insert a coding sequence outside the T-borders which, whether or not in cooperation with genes from the host plant or genes cotransferred, are toxic to plants so that there is counterselection for plants with superfluous vector-DNA.</p>		

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

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(ii) TITLE OF INVENTION: Novel plasmids for plant transformation and method for using same.

(iii) NUMBER OF SEQUENCES: 18

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: EP 97201990.5  
(B) FILING DATE: 30-JUN-1997

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GATGGCAAAC GCTAATAGG G

21

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GAGCGGGTTT AACCTACTTC C

21

10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

20

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTGTTGTCGA CGGCTGGCTG GTGGCAGGAT ATATTGTGG

39

30

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA

40

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCAGCCGTCG ACAACAGCTC CCCGACCGGC AGCTCGGC

38

50

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

5 (iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

10

TCGACGGGGT GCCTGGGGCG GTGGCGGGGC GGGCCGGGGC GTGC

44

(2) INFORMATION FOR SEQ ID NO: 6:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

30

TCGAGCACGC CCCGGCCCCG CCCGCCACCG CCCAGGCAC CCCG

44

(2) INFORMATION FOR SEQ ID NO: 7:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

45

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

50

TTCTCGAGAG GGTCGGTTAC TCG

23

(2) INFORMATION FOR SEQ ID NO: 8:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTGTCGACTC CAAGACGCCG AGC

23

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AATTCAGATC TCCATGGATC GATGAGCT

28

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CATCGATCCA TGGAGATCTG

20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GATCGGTCGA CC

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GACGCTAAAG GCAAACCTGA TTC

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTAACCTACT TCCTTTGGTT CCG

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

AGCGGCCCAT TTTAGTAGAC GGG

23

5 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

20 ATCAATTCGA ACCCCAGAGT CCCGTCCA

28

(2) INFORMATION FOR SEQ ID NO: 16:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

40 CTGGATCGTT TCGCATGATT G

21

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

45 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50 (iii) HYPOTHETICAL: NO

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CCAACTTATC AGTGATAAAG AATCCGC

27



(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GAGATCAGAT TGTCGTTTCC CGCCTTC

27